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Response of Soil Fungi and Soil and Plant Parameters to Sewage Sludge and Sawdust Amended Coal Mine Spoils

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The spoil fungal population was not significantly influenced, under field conditions. The differences in isolates from the sludge, sawdust, and nonamended treated spoils may be larger in the amended treatments than in the nonamended treatments when adequate moisture becomes available, and especially in the surface few inches of spoil than at the lower depths. The chemical analysis of the amended spoils, particularly in the sewage sludge treatment, showed increases in the concentrations of Cl^-, $NO_3^- - N$, and K^+.

Under greenhouse conditions, *Sorghum bicolor* yields were significantly higher when treated with sewage sludge than with any other treatment. Sawdust treatment had no significant effect on plant yields, probably because of the low nutrient levels from this particular amendment.

Keywords: Mine spoil, reclamation, mulch, *Sorghum bicolor*, soil microorganisms

Introduction

Most coal mine spoils from the Southwest contain high levels of soluble salts or exchangeable sodium, or both, which restrict plant growth (Aldon 1978, Rai et al. 1975, Sandoval and Gould 1978, USDA Forest Service 1979). In addition, these spoils may be low in mineralizable carbon, lack essential plant nutrients, and have a low or inactive microbial population (Fresquez and Lindemann 1982b, 1983).

Applications of organic materials usually improve soil productivity of surface-mined lands. But, because of the unfavorable environment, mainly the low precipitation inherent of this area, organic materials applied to spoils may decompose slowly. Attempts to assess the potential of organic amendments as a revegetation practice must consider the changes in soil chemical and physical properties, as well as changes in the soil biological community. Fluctuations in microbial populations and activities, for example, have been shown to be helpful in determining the feasibility of revegetation in mine spoils (Fresquez and Lindemann 1982a, 1982b; Fresquez et al. 1983; Hersman and Klein 1979; Miller and Cameron 1976; Miller et al. 1979; Sorensen et al. 1981).

The intent of the research reported here was to evaluate the distribution of fungal genera that take place when organic amendments are applied to coal mine spoil material, and determine their influence on spoil chemical properties, and plant growth of a grass species.

Materials and Methods

Study Area

The field portion of this study was conducted at the San Juan coal strip mine, approximately 24 km west of Farmington, N. Mex., on an exposure of the Fruitland Formation (U.S. Geological Survey 1963). The dominant soil types consist of moderately deep-to-deep coarse tex-

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tured soils developed over and from sandy eolian or alluvial sediments (Gould et al. 1977). The climate is semiarid continental with high diurnal temperature variations and infrequent precipitation. The total precipitation recorded at the San Juan coal mine from September 10, 1977 to September 15, 1978 was 18 cm. Dominant shrub species associated with the area include fourwing saltbush (*Atriplex canescens*), shadescale (*Atriplex confertifolia*), Nevada Mormon-tea (*Ephedra nevadensis*), and winterfat (*Ceratiodes lanata*). The dominant grasses associated with the area are Indian ricegrass (*Oryzopsis hymenoides*), alkali sacaton (*Sporobolus airoides*), and galleta (*Hilaria jamesii*).

Field plots were established on a spoil overburden site of the mine on September 10, 1977. The experimental area (2 m wide and 12 m long) was divided into 24 1–m^2 plots. Holes were dug in the center of each plot, and plastic asbestos tubes (11 cm in diameter and 100 cm long) were inserted into the spoil material to a depth of 94 cm. Each tube was then filled with spoil material.

The top 4 cm of spoil material was amended with anaerobically digested sewage sludge (Las Cruces Municipal Landfill, Las Cruces, N. Mex.), and sawdust at 10 Mg/ha. Both amendments had been individually sieved (2 mm) before being mixed with spoil material in the tubes. Each amendment treatment and nonamended control was replicated eight times in a completely randomized design. The treatments were allowed to incubate undisturbed for 1 year.

In September 1978, the tubes were removed, were covered at both ends, and were transported to the laboratory. The contents from the tubes were aseptically removed in two increments of 0-30 cm, and 30-100 cm. Samples were thoroughly mixed, subsampled, placed in sterile plastic bags, and were immediately stored in a refrigerator at 4°C. At that time, a composite spoil sample from both depths in each treatment was taken for chemical analysis by the New Mexico State University Soil and Water Testing Laboratory. All methods of analysis have been described previously (Fresquez and Lindemann 1983).

A plant tissue burial technique, similar to that used by Miller et al. (1979), was used for the spoil organism inventory. Two sterile sections of alfalfa straw (3 cm each in length) were placed in a petri dish and covered with approximately 25 grams of the spoil sample. The samples were moistened and were left to allow colonization of the straws by the organisms. One straw section was left in the spoil material 7 days, and the other for 30 days. At the end of each period, the alfalfa sections were removed, were washed free of adhering soil particles with sterile water, were cut evenly into three pieces, were plated on carrot agar medium (Staffeldt 1951), and were allowed to incubate for 3 days at ambient room temperature. Fungal identifications were classified as to their generic levels with the aid of various taxonomic guides (Barnett and Hunter 1972, Barron 1968, Gilman 1968). Variation in occurrence of fungal genera among treatments, soil depth and incubation time was analyzed using log linear models (Bishop et al. 1975). Soil parameters were analyzed using a two way analysis of variance. Significance was assessed using $\alpha = 0.05$.

Preliminary data from the field study suggested that a longer incubation period and/or more moisture may have been needed to fully determine treatment effects, in terms of nutrient mineralization. For these reasons, a greenhouse experiment was conducted on the remaining spoil material from the 0- to 30-cm depth of the amended and nonamended sections.

In this experiment, spoil material (4.2 kg per pot) from each treatment replicate was placed in a 20- x 18-cm (height x top diameter) cellophane lined plastic pot. Pot placement in the greenhouse was similar to the plot design in the field study. The pots of spoil were seeded to grain sorghum (*Sorghum bicolor* (L.)) Moench No. 2281 (New Mexico Department of Agriculture, Las Cruces), and seedlings were thinned to 10 plants per pot. The pots were maintained near field capacity (200 to 300 ml tapwater was added every other day to maintain 0.30 atm. matrix water potential) using 30 cm tensiometers installed 14 cm into the soil. Daily greenhouse temperatures ranged from 32°C days to 22°C nights.

After approximately 6 weeks of plant growth, the plants were harvested by removing the topgrowth 6 mm above the crown of the plant. Topgrowth was dried for 6 minutes at a high temperature setting using a 1.5 cubic foot microwave oven. Samples were then weighed, and weights were recorded (grams per plant). Immediately after removal of the topgrowth, subsamples of spoil from each pot were taken with a soil probe for soil chemical analysis. Data were analyzed using analysis of variance or t tests. Significance was assessed at $\alpha = 0.05$.

Results and Discussion

The indigenous spoil fungal population was not significantly influenced by treatment, demonstrated by the fact that the number of genera and the number of isolates from sludge, sawdust, and nonamended treated spoils were very similar (tables 1 and 2).

Normally, the number and types of microorganisms increase with the added application of organic matter. However, soil water has a direct effect upon the abundance and functions of fungi (Alexander 1979). The total precipitation recorded during this study period was only 18 cm (average per month was 1.51 cm with no major rain events recorded). It is suspected that when adequate moisture becomes available, both the genera diversity and the occurrences of individual isolates in the amended treatments may be wider. The introduction of new genera by amendment applications is likely. This seems to have been the case where *Mucor* appeared in the sludge treatment, while not appearing in either the sawdust or the nonamended material.

A significant interaction was observed between the number of isolates and soil depth (table 3). Alexander (1979) noted that the influence of depth upon the abundance and distribution of fungi is associated mainly with the concentration of organic matter and/or the composition of the soil atmosphere. Because no new substrates were available for an energy source by the fungi in the

Table 1.—Percentage frequency of occurrence of fungi isolated on straw traps after seven days (depth in cm)[1]

Genus	No amendment		Sludge		Sawdust	
	0—30	30—100	0—30	30—100	0—30	30—100
Actinomucor	0	13	13	0	0	13
Alternaria	100	13	100	25	100	13
Aspergillus	0	38	0	38	25	25
Aureobasidium	0	0	0	0	0	13
Cephaliophora	0	0	13	0	0	0
Cephalosporium	0	0	38	13	13	0
Chaetomium	50	13	0	38	38	75
Cladosporium	0	0	13	0	13	0
Cunninghamella	38	13	38	38	13	50
Curvularia	38	13	88	25	75	25
Fusarium	88	25	38	25	88	63
Gliocladium	38	75	0	88	25	100
Gliomastix	25	13	13	13	13	0
Histoplasma	13	25	75	0	50	0
Memnoniella	0	0	0	0	0	0
Mucor	0	0	88	13	0	0
Penicillium	63	88	63	75	63	63
Phoma	13	0	13	0	38	0
Pyrenochaeta	13	0	0	0	0	0
Rhinocladiella	0	0	0	0	13	0
Sclerotium	50	13	63	0	50	0
Sphaeropsidales	0	13	13	25	25	0
Stachybotrys	13	0	38	0	38	13
Streptomyces	38	63	38	63	75	63
Tetracoccosporium	50	0	75	0	38	25
Trichoderma	0	0	0	0	0	0
Trichurus	0	25	0	13	13	25
Verticillium	25	0	38	38	13	25
Totals of genera	16	15	19	15	21	15

[1]*Percentage frequency of occurrences* $= \frac{\text{number of occurrences of a fungal group x 100}}{\text{total number of plated straw = (8)}}$

nonamended treatments, the requirement for O_2 probably explains the higher number of isolated genera in the upper sections. Organisms that were encountered more in the 0- to 30-cm than in the 30- to 100-cm depth, regardless of applied treatment and time intervals between incubation periods, included *Alternaria, Curvularia, Fusarium, Histoplasma, Penicillium, Sclerotium, Streptomyces*, and *Verticillium*. Those genera that were found to be associated more with the lower 30- to 100 cm depth, include *Aspergillus, Chaetomium, Cunninghamella, Gliocladium, Penicillium, Streptomyces* and *Verticillium*. Organisms like *Penicillium, Streptomyces*, and *Verticillium* were observed at both depths. *Streptomyces* has been reported (Alexander 1979) to have a greater tolerance of CO_2 at the lower depths, while *Penicillium, Verticillium* and others that were found at both depths may have the ability to extend part of their mycelium from the surface sections to the lower sections.

Concentrations of major cations and anions in treated spoils after 1 year of field incubation tended to increase from the 0- to 30-cm depth to the lower 30-to 100-cm depth, although most were nonsignificant (table 4). Ions that were found to be significantly different among the treatments and for depths in the treatments were Cl^-, K^+, and $NO_3^- - N$. The sludge treatment contained the highest Cl^- and $NO_3^- - N$ concentrations throughout the profile, and the values were significantly higher in the 0- to 30-cm sections than in either the sawdust or the nonamended control. The 0- to 30-cm and 30- to 100-cm depths in the sawdust treatment did not differ significantly in Cl^- concentrations, while in the sludge treatment and control, there was a difference between the two depths. The sludge treatment had the highest concentrations of K^+ in the 0- to 30-cm depth, which was significantly higher than in the sawdust treatment or the nonamended control.

The chemical analyses of the amended and nonamended spoil materials after the greenhouse pot study with *S. bicolor* showed significantly higher concentrations of Cl^- and HCO_3^- in the sludge treated spoil materials, compared to the other treatments (table 5). The sawdust treatment had significantly lower values of SO_4^{2-} concentrations, while the nonamended control showed significantly higher values for K^+ and $NO_3^- - N$. The high $NO_3^- - N$ contents found in the nonamended spoil treatment compared to the low values observed in the sludge treatment after the harvest of the *S. bicolor* (table 5), and the higher plant production in the sludge treatment suggests that the sludge treatments provided a better plant growth medium, and that *S. bicolor* used $NO_3^- - N$ for growth. The low plant productivity on the nonamended spoil treatment and the sawdust treatment

Table 2.—Percentage frequency of occurrence of fungi isolated on straw traps after 30 days (depth in cm)[1]

Genus	No amendment		Sludge		Sawdust	
	0—30	30—100	0—30	30—100	0—30	30—100
Actinomucor	0	0	13	0	0	0
Alternaria	88	13	100	38	100	25
Aspergillus	0	38	0	25	0	38
Aureobasidium	0	0	0	0	0	0
Cephaliophora	0	0	0	0	0	0
Cephalosporium	13	0	25	13	25	0
Chaetomium	13	13	0	63	13	63
Cladosporium	13	13	0	38	0	13
Cunninghamella	13	38	25	38	13	13
Curvularia	63	0	63	0	88	0
Fusarium	100	38	25	25	88	50
Gliocladium	50	100	25	88	25	75
Gliomastix	25	13	0	13	0	0
Histoplasma	13	0	13	13	0	13
Memnoniella	0	0	0	0	0	0
Mucor	0	0	75	0	0	0
Penicillium	50	50	50	88	75	75
Phoma	13	0	0	0	38	0
Pyrenochaeta	13	0	0	0	0	0
Rhinocladiella	13	0	0	0	0	0
Sclerotium	38	0	38	13	13	0
Sphaeropsidales	25	0	13	13	63	0
Stachybotrys	38	25	25	13	38	0
Streptomyces	38	100	63	88	13	88
Tetracoccosporium	38	0	25	0	75	0
Trichoderma	0	0	0	0	0	13
Trichurus	0	13	13	25	25	38
Verticillium	38	25	38	38	38	63
Totals of genera	20	13	17	17	16	13

[1]*Percentage frequency of occurrences* $= \dfrac{\textit{number of occurrences of a fungal group x 100}}{\textit{total number of plated straw} = (8)}$

Table 3.—Total number of isolates of fungi as affected by spoil amendment (depths in cm)

	Treatments			
	No amendment	Sludge	Sawdust	Total[1]
7 days				
0-30	52[2]	68	65	185[3]
30-100	35	42	47	126
30 days				
0-30	55	50	58	163
30-100	38	50	45	133

[1]*Totals based on depth and time.*
[2]*Differences associated with amendment and incubation period were not significant ($p \leq .05$).*
[3]*Significant difference was associated with soil depth ($p \leq .05$).*

suggest that $NO_3^- - N$ was not used by the plants. The sawdust treatment, like the sludge treatment, also had a significant reduction in $NO_3^- - N$ levels after the harvest of S. *bicolor*; but in this case, the $NO_3^- - N$ was probably assimilated by the microflora.

Significantly higher values for the heavy metals Cu, Pb, and Zn were evident in the sludge treatment, while Mn in the sludge treatment was significantly lower than in the sawdust or the nonamended control (table 5). The concentrations of Co and Fe were not found to be significantly different among any of the treatments.

The EC, SAR, $NO_3^- - N$ and HCO_3^- values decreased significantly in all amended treatments, as a result of the application of water and the growth of plants (table 6). Although some decomposition was apparent, it appears that the 6-week greenhouse incubation period was not long enough to fully mineralize these particular organic amendments. In another similar greenhouse study, Fresquez and Lindemann (1983) reported significant increases in nutrients in sludge and alfalfa amended spoils in an incubation period of approximately 24 weeks.

The dry weights of S. *bicolor* grown on the sludge treated spoil material were significantly greater (0.05 probability level by Duncan's Multiple Range Test) than plant growth in the sawdust or the nonamended control:

Spoil treatments	Plant dry weights
	grams per plant
No amendment	0.27b
Sludge	1.37a
Sawdust	0.32b

Table 4.—Chemical analyses of coal mine spoil 1 year after applying spoil amendments (depth in cm)

Chemical entity[1]	No amendment		Sludge		Sawdust	
	0–30	30–100	0–30	30–100	0–30	30–100
EC	12.0a[2]	13.8a	11.8a	13.8a	11.7a	13.3a
SAR	42.5a	48.8a	40.4a	45.0a	39.5a	43.9a
Na	158.4a	192.5a	157.7a	177.0a	152.1a	175.8a
Ca	19.5a	21.3a	20.8a	20.3a	20.5a	721.7a
Mg	8.2a	9.8a	8.8a	10.6a	9.3a	10.5a
K	0.78b	0.75c	0.83a	0.72d	0.75c	0.72d
Cl	1.3d	1.8c	2.7a	2.0b	1.5c	1.6c
SO_4	168.3a	186.5a	150.3a	166.8a	157.5a	176.4a
HCO_3	2.4a	2.9a	2.3a	2.3a	2.4a	2.8a
NO_3	2.0c	2.8b	3.5a	3.1b	2.0c	3.0b

[1]*EC is expressed as mmhos/cm, while cations, anions, and nitrogen are expressed as meq/L.*
[2]*Means within the same horizontal row followed by the same letter are not significantly different at the 0.05 probability level by Duncan's Multiple Range Test.*

Table 5. —Chemical analysis of the amended spoil materials after the greenhouse pot study

Chemical entity[1]	Spoil treatments		
	No amendment	Sludge	Sawdust
EC	10.8a[1]	10.5a	10.1a
SAR	34.8a	4.5a	35.9a
Na	134.6a	134.5a	137.5a
Ca	22.1a	21.8a	21.4a
Mg	7.9a	8.6a	8.0a
K	0.82a	0.78b	0.75b
Cl	5.4b	7.2a	5.2b
SO_4	157.9a	156.8a	145.5b
HCO_3	1.4b	1.6a	1.4b
NO_3	3.0a	0.40b	0.26b
Cu	1.9b	2.7a	1.9b
Co	0.30a	0.28a	0.33a
Fe	1.7a	1.6a	1.6a
Mn	0.85a	0.57b	0.79a
Pb	0.60b	1.40a	0.67b
Zn	0.13b	0.39a	0.14b

[1]*EC is expressed as mmhos/cm; the cations, anions, and nitrogen are expressed as meq/L; and the heavy metals are expressed as ppm.*
[2]*Means within the same horizontal row followed by the same letter are not significantly different at the 0.05 probability level by Duncan's Multiple Range Test.*

The dry weights from the sawdust treatment and the control did not differ significantly. The most obvious factor for the low plant yields noted in the sawdust treatment is the low nutrient levels, mainly available nitrogen. The addition of more carbon in the sawdust treatment probably only widened the C:N ratio initially present in this spoil material, resulting in most of the available nitrogen and possibly other nutrients being tied up by the microflora, instead of being utilized by the plant.

Conclusions

Soil fungi generally require at least moderate moisture levels for growth (Harris 1981). Their capacity for decomposition proccesses may be appreciably reduced when moisture is low (Sommers et al. 1981). This may be one reason why there were no significant increases found in either genera or number of isolates with the added application of organic matter. The only significant effect observed resulted from spoil depth. More fungi were found in greater generic frequencies in the 0- to 30-cm depth than at the 30- to 100-cm depth. It appears that, at the San Juan coal mine, the greatest potential for decomposition processes is on the surface few inches when moisture is available.

The applications of organic amendments were shown to be beneficial in terms of supplying inorganic nutrients to the spoil. Concentrations of $NO_3^- - N$, Cl^-, and K^+ were found to be highest in those spoil materials treated with the sewage sludge. The greenhouse data revealed that the spoil material amended with the sewage sludge seemed superior to other treatments in terms of producing higher yields of Sorghum *bicolor*. The sawdust amendment, in contrast, did not seem to add any appreciable plant nutrients.

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Table 6.—Mean comparisons of the water soluble extracts of the 0–30 cm depths before planting and after harvest of *Sorghum bicolor* (L.) Moench

Chemical Entity	No amendment		Sludge		Sawdust	
	Before	After	Before	After	Before	After
EC	12.00	10.80	11.80	10.50*	11.70	10.10*
SAR	42.5	34.80*	40.40	34.5*	39.50	35.90*
Cl	1.30	5.40*	2.70	7.20*	1.50	5.20*
HCO_3	2.40	1.40*	2.30	1.60*	2.40	1.40*
SO_4	168.30	157.90	150.30	156.80	157.60	145.50
NO_3	2.00	3.00	3.50	0.40*	2.00	0.26*
K	0.78	0.82	0.83	0.78	0.75	0.75
Ca	19.50	22.10*	20.80	21.80	20.50	21.40
Mg	8.20	7.90	8.80	8.60	9.30	8.00*
Na	158.40	134.60*	154.70	134.50*	152.10	137.50

**Significant according to t test comparing before and after measurements ($p = 0.05$).*

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